Biotic and Abiotic Factors Limiting Efficacy of Bt Corn in Indirectly Reducing Mycotoxin Levels in Commercial Fields

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ABSTRACT Incidence of insect damage, and association of insect damage with mycotoxigenic corn ear molds and mycotoxins was examined in commercial fields of Bt and non-Bt hybrids of different backgrounds in Illinois in 1998 and 1999. Nearly 50% *Helicoverpa zea* (Boddie) infestation sometimes occurred in Bt hybrids that express high levels of the protein in silks and kernels. Damage by European corn borer, *Ostrinia nubilalis* Hübner, was uncommon, even in non-Bt ears. Levels of total fumonisins were generally less (15- to 1.8-fold) in Bt versus non-Bt hybrids at the same site, with some significant differences. There were several instances where there were no significant differences in fumonisin levels between low/no Bt kernel hybrids and Bt hybrids that produced high levels of the protein in the kernel and silk tissue. However, significant correlations were often noted between numbers of insect-damaged kernels and total fumonisin levels, especially in 1998, suggesting in these cases that reducing insect damage was still reducing fumonisin levels. There was variability between the correlation coefficient for numbers of insect damaged kernels and fumonisin levels at different sites for the same year, different hybrids at the same site, and the same hybrid for different years. Although reductions in fumonisins in Bt hybrids were more limited than reported in the past, planting the Bt hybrids still appears to be a useful method for indirectly reducing mycotoxins in corn ears.

KEY WORDS *Bacillus thuringiensis, Ostrinia, Helicoverpa, Fusarium,* fumonisin

SEVERAL HUNDREDS OF millions of dollars in losses occur each year in the United States from mycotoxins, the toxins produced by ear molds in corn (CAST 1989, USDA 1999). Insects and their damage frequently increase levels of ear molds and their mycotoxins in corn (review, Dowd 1998). Reducing insect damage through application of insecticides also reduces mycotoxin levels, but the number of applications required is typically not economically feasible (review, Dowd 1998).

Bt corn hybrids can provide highly effective control of European corn borers, *Ostrinia nubilalis* Hübner (e.g., Munkvold et al. 1997, Pilcher et al. 1997b, Dowd 2000). When *O. nubilalis* is the predominant insect pest present, Bt corn has substantially reduced levels of fumonisins in small plots (Munkvold et al. 1999, Dowd 2000) and larger fields under natural conditions (Dowd 2000). Efficacy of the Bt hybrids that express the protein at high levels throughout the plants against corn earworms, *Helicoverpa zea* (Boddie), ranges from nearly complete control (Rice and Pilcher 1998), to <90% (Gould 1998), to only limited effectiveness (Dowd et al. 1998a, 1999a; Dowd 2000). In previous studies, the presence of *H. zea* in Bt hybrids appeared to limit effectiveness of Bt hybrids in indirectly reducing mycotoxins in 0.4-ha fields in Illinois in 1998 (Dowd 2000).

The current study was designed to investigate potential reductions in mycotoxins such as fumonisins in Bt corn compared with non-Bt corn hybrids of interest to growers in large commercial fields for the first time. Whenever possible, several biotic and abiotic factors that may influence efficacy such as location, year-to-year variation, hybrid type, and damage caused by different insect species were determined. During evaluations of Bt hybrids for insect control and indirect reduction of mycotoxins in commercial fields, high levels of *H. zea* were present in test areas in both 1998 and 1999. The current report indicates that despite high correlations between insect damage and fumonisin levels for some hybrids, reduction in fumonisin levels is much less than has been reported when *O. nubilalis* is the predominant insect ear pest. Also included is information indicating that some hybrids examined did not have a high correlation with insect damage and fumonisin levels and thus would be less useful for indirectly reducing mycotoxins in corn. Summaries of these studies have appeared previously (Dowd et al. 1998a, 1999a).

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Materials and Methods

Plants. With one exception, plants sampled in 1998 and 1999 came from commercial fields in Mason

County, IL. Plants were grown using conventional practices, which included center pivot irrigation. Sizes of fields are listed below (see *Results*) and reflect the combined area planted to strips of the two hybrids being compared at that location. Because of grower preferences and seed availability, isogenic Bt and non-Bt hybrid pairs were not always available in the same field. However, either a non-Bt hybrid (NKN69R1, NK4494, P3394) or a Bt hybrid that expressed low levels in the kernels and silks (NKMax454, event 176) was available in the same field for comparison with a Bt hybrid that expressed the protein throughout the plant (NK7639Bt, NK6800Bt, NK7070Bt - all Bt 11 events, P33VO8 - mon810 event). Past results have indicated NKMax454 fumonisin levels typically are not much different from fumonisin levels in NK6800 or P3394 when planted at the same site in this area of Illinois (Dowd 2000). Specific combinations at different sites and years are presented in *Results.* Hybrids were planted in eight-row alternating strips across the fields (which were approximately square) except at Kilbourne, IL, in 1999, when hybrids were planted in alternating four-row strips. All hybrids were planted in rows 76.2 cm apart, with 58,000 plants/ha at the Manito site and 65,250 plants/ha at the other sites.

Sampling. All fields were sampled in an X pattern as described previously (Dowd et al. 2000). Samples were taken at the "arm tips" and center of the X. The "arm tip" samples were about one-fifth of the way into the field from each corner, and the center sample was in the approximate center of the field. Both milk stage (R3, ca. 21 d after pollination, Ritchie et al. 1989), and harvest stage (<20% moisture) samples were taken. Twenty-five ears were to be picked from every other plant in center rows at each sample position in the respective field, and milk stage ears were individually bagged. To avoid confounding data, ears that were obviously undersized because of occasional plant crowding, or ears that were vertebrate damaged or damaged by the irrigation pivot, were not sampled. Samples were taken to the laboratory, and insect species present (including beneficials) and occurrence and numbers of kernels damaged by different insect species were recorded. Milk stage ears were individually examined in a white dish pan, and damage could be readily associated with the associated insect species present. Although insects were typically absent from harvest stage ears, characteristic types of damage and frass could be used to distinguish damage from different insect species (see Dowd 1998 and Dowd 2000 for photos and descriptions distinguishing different types of insect damage). Occurrence and numbers of symptomatically molded kernels infected by ear rot fungi such as *Aspergillus flavus* Link or *Fusarium* species were also determined, as indicated by visible mold or symptomology such as kernel streaking (Dowd et al. 1999b) and based on earlier publications (Shurtleff 1980). Because of the association of high levels of fumonisins with kernels that were damaged early (milk to soft dough, R3 to early R4 of Ritchie et al. 1989), as indicated by discolored pericarps (Dowd et al. 1999b), the age of kernel damage was also recorded.

Mycotoxin Analysis. Analysis for aflatoxins and fumonisins was performed by HPLC as described previously (Dowd et al. 1999b, Dowd 2000). Limits of detection were 0.1 ppm for fumonisins B_1, B_2, and B_3, and 0.1 ppb for aflatoxins B_1, B_2, G_1 and G_2. Insect damaged kernels of commercial hybrids may have levels of fumonisins from several hundred to >1,000 ppm (Dowd et al. 1999b) or aflatoxins >50,000 ppb (Dowd et al. 1998b). Because of this mycotoxin distribution, insect-damaged kernels were separated and analyzed apart from the undamaged kernels to increase precision and sensitivity. Mycotoxin levels calculated for the entire sample were based on relative weights of the separated kernels (Dowd et al. 1999b, Dowd 2000). Kernels of all ears from each sample position were combined for analysis. Analyses were run by Romer Laboratories (Union, MO).

Statistical Analysis. Significant differences in incidence data were determined by chi-square analysis (SAS PROC Freq) because of frequent non-normal distribution across the fields, as described previously (Dowd et al. 1999b). Significant differences in numbers of kernels damaged per ear or fumonisin levels were determined by analysis of variance (ANOVA). Mycotoxin values were transformed for analysis using a log transformation. Significant correlations between mycotoxin levels and numbers of insect-damaged or symptomatically infected kernels were determined using SAS PROC Reg, option Corr (SAS Institute 1987).

Results

The incidence of *O. nubilalis* was generally low in all hybrids in both years. Incidence of *O. nubilalis* at milk stage was only >10% at the Easton site in 1998 (Table 1). However, the Bt hybrids that expressed the gene at high levels in ears and silks were free of *O. nubilalis* damage at Easton in 1998.

In contrast, *H. zea* incidence at milk stage was typically >10% in all years at all sites, and was up to 69% on the non-Bt NKN69R1 hybrid in 1998. Incidence of *H. zea* at milk stage was not significantly reduced in Bt versus non-Bt hybrids, even those that expressed the gene throughout the plant, except for non-Bt NKN69R1 versus low Bt NKMax454 at Manito in 1998 ($P = 0.000$, $\chi^2 = 33.835$).

When damage occurred at the same sample site, the numbers of kernels damaged on ears at milk stage by *H. zea* was significantly less in Bt hybrids that expressed the gene at high levels in kernels and silks compared with non Bt hybrids or Bt hybrids that expressed low levels of the gene in kernels and silks in nearly all cases ($P < 0.01$ at least) (Table 2). Reductions in numbers of kernels damaged per milk stage ear were often around three-fold (Table 2).

Samples of Bt corn ears from some sites had ears with relatively large numbers of kernels slightly damaged by *H. zea* larvae ("railroading"), a phenomenon which was not seen with non or low Bt ears. This

Table 1. Percentage incidence of insects on Bt and nonBt hybrid corn ears at milk stage in commercial fields in 1998 and 1999

Field	Hybrid	*O. nubilalis*	*H. zea*	*S. frugiperda*	Total caterpillar	Sap beetles	Stink bugs	Predators[a]
				1998				
Manito	NKN69R1	2.6	69.2a	0.0	70.9a	8.5a	0.0a	NR
3.2 ha	NKMax454	0.0	32.0b	0.0	32.0b	8.6a	0.0a	NR
Easton	NK4494	40.0a	12.0a	3.2a	52.0a	20.0a	0.0a	NR
20 ha	NKMax454	32.8ay	12.8ay	5.6ay	50.4ay	17.6ay	0.0a	NR
Easton	NK6800Bt	0.0az	20.8az	0.8ay	21.6az	7.2az	1.6a	NR
20 ha	NK7639Bt	0.0az	24.0az	4.0ay	26.4az	11.2ay	12.8b	NR
				1999				
Kilbourne	P3394	5.6a	16.0a	0.0a	20.8a	24.8a	7.2a	33.6a
0.8 ha	P33V08	0.0b	13.6a	0.0a	13.6b	13.6b	2.4a	28.0a
Bath	NKMax454	4.0a	23.2a	2.4a	28.8a	21.6a	0.0a	35.2a
28 ha	NK6800Bt	0.0b	18.4a	0.0a	18.4b	4.8b	0.0a	31.2a
Easton	NKMax454	1.6a	9.6a	0.8a	12.1a	10.4a	0.8a	42.4a
16 ha	NK7070Bt	2.4a	5.6a	0.0a	8.1a	2.4b	0.8a	33.6a

Fields at Easton in 1998 were adjacent, and y/z statistical comparisons involve max454 compared with the two NK hybrids. Hybrids NKN69R1, NK4494, and P3394 express no Bt protein, hybrid NKMax454 expresses low levels of the Bt protein in the silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 express high levels of the Bt protein throughout the plant. Total caterpillar refers to combined incidence for *O. nubilalis*, *H. zea*, and *S. frugiperda*, which are not necessarily additive to the occurrence of more than one species on the same ear. In some cases numbers of ears collected were slightly higher or lower target collection numbers, and incidence values reflect actual numbers collected. Values in columns of like fields and years followed by different letters are significantly different at $P < 0.05$ by chi-square analysis.

[a] NR, not reported because of low numbers.

railroading occurred in 13.0 and 14.3% of *H. zea*-damaged ears of NK6800Bt and NK7639, respectively from Easton in 1998, and from 30.4% of *H. zea* damaged NK6800Bt ears from Bath in 1999. Only the ears from Bath showed significantly higher ($P = 0.001$, $\chi^2 = 10.199$) railroading than the low Bt ears (0% railroading) at Bath.

Fall armyworms, *Spodoptera frugiperda* J.E. Smith, were relatively uncommon, but did occur at low levels at some sites in both years.

The sap beetles (Coleoptera: Nitidulidae) present on milk stage ears were primarily *Carpophilus lugubris* Murray and *Carpophilus antiquus* Melsheimer. Sap beetle adult incidence was often reduced in Bt hybrids

Table 2. Numbers of kernels damaged per ear by insects on milk stage Bt and nonBt hybrids in commercial fields in 1998 and 1999

Field	Hybrid	*O. nubilalis*	*H. zea*	Sap beetles	Stink bugs
			1998		
Manito	NKN69R1	20.0 ± 5.0	28.8 ± 1.8a	2.4 ± 0.3a	—
3.2 ha	NKMax454	—	18.0 ± 2.4b	2.3 ± 0.4a	—
Easton	NK4494	13.1 ± 1.2a	39.4 ± 4.4a	1.7 ± 0.2a	—
20 ha	NKMax454	8.4 ± 1.9a	33.4 ± 3.5ay	2.4 ± 0.4ay	—
Easton	NK6800Bt	—	12.1 ± 2.6az	2.3 ± 0.9ay	ND[a]
20 ha	NK7639Bt	—	12.6 ± 2.0az	2.8 ± 0.6ay	ND[a]
			1999		
Kilbourne	P3394	11.0 ± 2.6	27.5 ± 3.2a	1.2 ± 0.2	5.6 ± 1.1a
0.8 ha	P33V08	—	10.0 ± 3.6b	1.0 ± 0.0	7.3 ± 1.5a
Bath	NKMax454	—	26.3 ± 2.3a	—	—
28 ha	NK6800Bt	—	9.9 ± 1.3b	2.0	—
Easton	NKMax454	5.0	20.7 ± 4.3a	1.0 ± 0.0	1.0
16 ha	NK7070Bt	3.0	6.8 ± 1.8b	1.4 ± 0.3	1.0

Fields were adjacent at Easton in 1998. Hybrids NKN69R1, NK4494, and P3394 expressed no Bt protein, hybrid NKMax454 expressed low levels of the Bt crystal protein in silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 expressed high levels of the Bt protein throughout the plant. Values are means ± SEs for ears where insects were found. Kernel values are based on filled kernels only. Single values listed (without standard errors) indicated only one individual data point occurred, and dash marks indicate no values occurred because the insect was not present. Values in columns of like fields in like years followed by different letters are statistically different at $P < 0.05$ by ANOVA.

[a] Not determined.

Table 3. Percentage incidence of insects on Bt and nonBt hybrid ears at harvest stage in commercial fields in 1998 and 1999

Field	Hybrid	Early caterpillar	Late caterpillar	Total caterpillar	Sap beetle	Stink bug	Popped
			1998				
Manito	NKN69R1	44.0a	0.8a	44.8a	66.4a	4.0a	0.0a
3.2 ha	NKMax454	16.8b	0.0b	16.8b	30.4b	10.4b	0.0a
Easton	NK4494	34.4a	36.0a	64.8a	35.2a	12.0a	0.8a
20 ha	NKMax454	23.4ay	36.3ay	58.1ay	29.0ay	4.8by	0.8ay
Easton	NK6800Bt	7.3az	46.0ay	48.4ay	4.0az	4.8ay	9.7az
20 ha	NK7639Bt	18.3by	24.6bz	37.3az	17.5bz	23.0bz	6.3az
			1999				
Kilbourne	P3394	14.4a	11.9a	24.6a	26.2a	50.8a	14.3a
0.8 ha	P33V08	6.4b	4.0b	9.6b	14.4b	58.4a	16.8a
Bath	NKMax454	24.0a	9.6a	32.0a	10.4a	22.4a	7.2a
28 ha	NK6800Bt	9.6b	10.4a	16.8b	7.2a	20.8a	9.6a
Easton	NKMax454	6.4a	15.2a	20.8a	34.4a	34.4a	0.8a
16 ha	NK7070Bt	0.8b	2.4b	3.3b	45.5a	55.3b	4.9a

Fields at Easton were adjacent in 1998. Hybrids NKN69R1, NK4494, and P3394 express no Bt protein, hybrid NKMax454 expresses low levels of the Bt protein in the silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 express high levels of the Bt protein throughout the plant. Total caterpillar refers to combined incidence of early and late caterpillar damage on ears. Because of the occurrence of both types of caterpillar damage on the same ears, total caterpillar values are not necessarily additive from values for early and late caterpillar damage. Popped refers to kernels with split pericarps and visible expanded endosperms. In some cases, numbers of ears collected were slightly higher or lower that target collection numbers, and incidence values reflect actual numbers collected. Values in columns from like fields and years followed by different letters are statistically different at $P < 0.05$ by chi-square analysis.

that expressed high levels of the gene in kernels and silks compared with non-Bt or Bt hybrids that expressed the gene at low levels in kernels and silks in both years (Table 1). However, when sap beetle damaged occurred, the number of kernels damaged per ear at milk stage was generally not significantly different between hybrids of different types at the same site.

Predators were relatively uncommon on milk stage ears in 1998, and no significant differences in predator numbers between hybrids at corresponding sites were noted (data not shown). Predators were more common on milk stage ears in 1999, and there were no significant differences in the incidence of predators (which were mostly *Orius* spp.) between the different hybrid types at the different sites. Predator incidence varied as much among different non/low Bt hybrids as it did for non/low Bt versus high Bt hybrids at the same site. Even among different sites, predator incidence was relatively similar.

There is a demonstrated strong association between kernels that are damaged early (as indicated by discolored pericarps) and high levels of fumonisin (Dowd et al. 1999b), so data reported for harvest samples emphasizes these kernels. Incidence of early caterpillar damage at harvest was generally significantly less for Bt hybrids that expressed the gene at high levels in kernels and silks compared with hybrids that expressed low or noBt in kernels and silks (values ranging from $P = 0.04$, $\chi^2 = 4.202$ for Kilbourne in 1999 to $P = 0.000$, $\chi^2 = 67.617$ for Manito in 1998) (Table 3). Incidence of late damaged kernels (no discolored pericarp) also tended to be significantly less for high Bt hybrids compared with other hybrids at the same site. However, the number of remaining kernels damaged per ear when damage was present was generally the same for high Bt versus low or non-Bt hybrids (Table 4). Incidence of sap beetle damage at harvest was generally lower in low versus non-Bt and high versus low Bt hybrids in 1998 but not 1999 (Table 3). In most cases, when sap beetle damage was present, the number of damaged kernels per ear was not significantly different for hybrids planted at the same site. Damage by *Euschistus* spp. stink bugs (Hemiptera: Pentatomidae, based on insects collected at milk stage), was relatively common in both years at all sites but showed no significant trend for non-Bt versus low Bt, low Bt versus high Bt, or non-Bt versus high Bt hybrids. There were no significant differences for incidence of popped kernels (kernels with split pericarps and visible expanded endosperms) for corresponding hybrids at the same site and year, except at Easton in 1998 for low versus high Bt hybrids ($P = 0.002$, $\chi^2 = 9.923$).

There was a trend for reduced incidence of symptomatic *Fusarium* spp. molded kernels from high Bt hybrids compared with low or non-Bt hybrids when incidence of damage by caterpillars or sap beetles exceeded 10%. Significant reductions in incidence of symptomatic *Fusarium*-molded kernels occurred for one high (NK6800Bt) versus low (NKMax454) Bt hybrid combination for sap beetles ($P = 0.001$, $\chi^2 = 11.511$) and caterpillars ($P = 0.018$, $\chi^2 = 5.605$) at Easton in 1998 and caterpillars alone ($P = 0.025$, $\chi^2 = 5.024$) at Bath in 1999.

Symptomatic *Fusarium* mold was rarely associated with stink bug damage for any hybrid in either year (Table 5). When symptomatic *Fusarium* mold was present, there were no significant differences in numbers of moldy kernels per ear for any corresponding hybrid pairs except NK7639Bt (higher) versus NK-

Table 4. Numbers of kernels damaged per ear by insects for Bt and nonBt hybrids at harvest in commercial fields in 1998 and 1999

Field	Hybrid	Early caterpillar	Total caterpillar	Sap beetle	Stink bug	Popped
			1998			
Manito	NKN69R1	14.0 ± 1.2a	14.0 ± 1.2a	5.9 ± 0.7a	3.8 ± 1.1a	—
3.2 ha	NKMax454	11.7 ± 1.2a	11.7 ± 1.2a	4.4 ± 0.5a	6.3 ± 1.2a	—
Easton	NK4494	16.3 ± 1.9a	11.0 ± 1.2a	3.3 ± 0.5a	7.2 ± 1.0a	1.0
20 ha	NKMax454	13.5 ± 1.8ay	8.8 ± 0.9ay	2.5 ± 0.3ay	9.5 ± 2.0ay	3.0
Easton	NK6800Bt	5.2 ± 0.0az	6.1 ± 0.9ay	1.6 ± 0.2ay	12.2 ± 3.4ay	2.1 ± 0.4
20 ha	NK7639Bt	6.1 ± 0.0az	10.2 ± 1.5bz	5.2 ± 0.2bz	10.0 ± 1.2ay	3.1 ± 0.8
			1999			
Kilbourne	P3394	6.8 ± 1.0a	7.4 ± 1.1a	2.5 ± 0.4a	5.0 ± 0.5a	3.8 ± 1.0a
0.8 ha	P33V0	8.9 ± 2.3a	8.4 ± 2.0a	2.6 ± 0.4a	5.8 ± 0.7a	4.2 ± 0.8a
Bath	NKMax454	8.9 ± 1.0a	10.4 ± 1.2a	2.5 ± 0.5a	5.6 ± 1.1a	3.6 ± 0.9a
28 ha	NK6800Bt	8.2 ± 1.9a	11.1 ± 2.2a	3.9 ± 0.8a	5.1 ± 0.7a	2.9 ± 0.5a
Easton	NKMax454	16.5 ± 2.0a	8.3 ± 1.9a	3.9 ± 0.8a	6.5 ± 1.1a	3.0 ± 2.0a
16 ha	NK7070Bt	16.0 ± 7.0a	10.6 ± 4.7a	2.9 ± 0.3a	8.0 ± 0.7a	1.2 ± 0.2a

Fields at Easton were adjacent in 1998. Hybrids NKN69R1, NK4494, and P3394 expressed no Bt protein, hybrid NKMax454 expressed low levels of the Bt crystal protein in silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 expressed high levels of the Bt protein throughout the plant. Values are means ± SE for ears where insect damage was found. Total caterpillar refers to combined numbers of early- and late- damaged kernels on an ear where ear damage occurred. Total caterpillar damage may have lower or higher mean values than early caterpillar damage depending on the numbers of early damaged relative to late damaged kernels. Popped refers to kernels with split pericarps and visible expanded endosperms. Values in columns of like fields and years followed by different letters are statistically different at $P < 0.05$ by ANOVA.

Max454 for both caterpillars ($F = 16.490$; df = 1, 14; $P = 0.001$) and sap beetles ($F = 46.2973$; df = 1, 12; $P = 0.000$), and NKN69R1 (higher) versus NKMax454 for caterpillars ($F = 5.067$; df = 1, 38; $P = 0.029$) in 1998 (Table 6).

Except for a few cases, fumonisin levels were not significantly different in non-Bt versus lowBt, low Bt versus high Bt, or non-Bt versus high Bt hybrids for any site or year, although there was a consistent trend for high Bt hybrids to have less fumonisin than low or non-Bt hybrids planted at the same site (Table 7).

Table 5. Percentage incidence of symptomatic *Fusarium* mold from different sources on ears of Bt and nonBt hybrids at harvest in commercial fields in 1998 and 1999

Field	Hybrid	Early caterpillar	Sap beetle	Stink bug	Popped
		1998			
Manito	NKN69R1	25.6a	27.2a	0.0a	—
3.2 ha	NKMax454	5.6b	11.2b	0.0a	—
Easton	NK4494	22.4a	12.0a	0.0a	0.0a
20 ha	NKMax454	8.1by	8.9ay	0.0a	0.0a
Easton	NK6800Bt	1.6az	0.0az	0.0a	0.0a
20 ha	NK7639Bt	5.6ay	4.0by	0.0a	0.0a
		1999			
Kilbourne	P3394	4.0a	18.3a	0.8a	3.2a
0.8 ha	P33V08	3.2a	8.8b	0.8a	4.0a
Bath	NKMax454	15.2a	5.6a	0.0a	1.6a
28 ha	NK6800Bt	6.4b	4.0a	0.0a	0.8a
Easton	NKMax454	5.6a	18.4a	1.6a	0.0a
16 ha	NK7070Bt	1.6a	18.7a	0.0a	0.0a

Fields at Easton in 1998 were adjacent. Hybrids NKN69R1, NK4494, and P3394 expressed no Bt protein, hybrid NKMax454 expressed low levels of the Bt crystal protein in silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 expressed high levels of the Bt protein throughout the plant. Popped refers to kernels with split pericarps and visible expanded endosperms. In some cases, numbers of ears collected were slightly higher or lower that target collection numbers, and incidence values reflect actual numbers collected. Values in columns of like fields and years followed by different letters are statistically different at $P < 0.05$ by chi-square analysis.

Table 6. Numbers of symptomatic *Fusarium* molded kernels per ear from different sources for Bt and nonBt hybrids at harvest in commercial fields in 1998 and 1999

Field	Hybrid	Early caterpillar	Sap beetle	Stink bug	Popped
		1998			
Manito	NKN69R1	7.6 ± 0.9a	3.8 ± 0.5a	—	—
3.2 ha	NKMax454	3.9 ± 0.5b	2.5 ± 0.5a	—	—
Easton	NK4494	3.6 ± 0.6a	2.0 ± 0.3a	—	—
20 ha	NKMax454	3.0 ± 0.5ay	1.5 ± 0.3ay	—	—
Easton	NK6800Bt	4.2 ± 1.0ay	—	—	—
20 ha	NK7639Bt	7.7 ± 1.3az	6.2 ± 0.7z	—	—
		1999			
Kilbourne	P3394	2.2 ± 0.4a	1.8 ± 0.2a	1.0 ± 0.0	1.8 ± 0.5a
0.8 ha	P33V08	2.0 ± 0.6a	1.5 ± 0.2a	2.0 ± 0.0	2.8 ± 1.1a
Bath	NKMax454	5.2 ± 0.8a	1.7 ± 0.4a	—	2.0 ± 1.0
28 ha	NK6800Bt	5.2 ± 2.2a	3.8 ± 1.2a	—	2.0 ± 0.0
Easton	NKMax454	7.0 ± 1.4a	4.0 ± 0.9a	2.3 ± 0.7	—
16 ha	NK7070Bt	11.0 ± 4.0a	2.8 ± 0.5a	—	—

Fields at Easton were adjacent in 1998. Hybrids NKN69R1, NK4494, and P3394 expressed no Bt protein, hybrid NKMax454 expressed low levels of the Bt crystal protein in silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 expressed high levels of the Bt protein throughout the plant. Values are means ± SE for ears where damage was found. Popped refers to kernels with split pericarps and visible expanded endosperms. Values in columns from like fields and years followed by different letters are statistically significant at $P < 0.05$ by ANOVA.

Table 7. Fumonisin levels in Bt and nonBt hybrid corn from commercial fields in 1998 and 1999

Field	Hybrid	Fumonisin, ppm	Fumonisin correlations[a]	
			Insect-damaged kernels	Symptomatic molded kernels
		1998		
Manito	NKN69R1	0.40 ± 0.15a	0.98*	0.98*
3.2 ha	NKMax454	0.022 ± 0.009b	0.009	0.45
			both 0.95*	both −0.32
Easton	NK4494	1.08 ± 0.38a	0.85*	−0.27
20 ha	NKMax454	0.74 ± 0.22ay	0.45	−0.31
			both 0.72*	both −0.32
Easton	NK6800Bt	0.37 ± 0.17ay	0.91*	0.08
20 ha	NK7639Bt	0.60 ± 0.12ay	0.90*	−0.38
		1999		
Kilbourne	P3394	0.50 ± 0.19a	0.56	0.21
0.8 ha	P33V08	0.16 ± 0.12b	(both)	
Bath	NKMax454	1.08 ± 0.49a	0.34	0.70
28 ha	NK6800Bt	0.42 ± 0.30a	0.99*	0.96*
Easton	NKMax454	0.89 ± 0.40a	−0.20	0.32
16 ha	NK7070Bt	0.14 ± 0.06b	0.43	0.31

Values in rows from like fields and years followed by different letters are statistically different at $P < 0.05$ by ANOVA for fumonisin levels. Hybrids NKN69R1, NK4494, and P3394 expressed no Bt protein, hybrid NKMax454 expressed low levels of the Bt crystal protein in silks and kernels, and hybrids NK6800Bt, NK7639Bt, NK7070Bt, and P33V08 expressed high levels of the Bt protein throughout the plant.

[a],* Correlations that are statistically significant at $P < 0.05$.

Significant correlations were more common between numbers of insect-damaged kernels and fumonisin levels in 1998 than in 1999, although NK6800Bt had correlations of 0.90 or above in both years ($P = 0.0109$ in 1998 and $P = 0.0105$ in 1999). Correlations between symptomatically molded kernels and fumonisin levels were lower than for insect damage and fumonisin levels in both years, although in some cases correlations were high (NK4494, $R = 0.98$, $P = 0.003$, for Manito in 1998; NK6800Bt, $R = 0.96$, $P = 0.002$, for Bath in 1999). No *Aspergillus flavus* Link or aflatoxins were detected in any samples.

Discussion

Types of Insect Damage. TheBt hybrids that express the gene at high levels in kernels and ears are very effective at preventing *O. nubilalis* damage of kernels (Dowd et al. 1997, 1998a 1999a; Munkvold et al. 1997, 1999; Pilcher et al. 1997b; Dowd 2000). However, the level of control of *H. zea* by the high Bt hybrids has been variable. Reports have described near complete control (Rice and Pilcher 1998, <90% control (Gould 1998) or little or no control (Dowd et al. 1998a, 1999a, Dowd 2000). A previous study (Dowd 2000) and the current study also indicate that although *H. zea* feeding is slowed down on Bt hybrids expressing high levels of the protein in the kernels (as indicated by lower numbers of damaged kernels at milk stage), incidence is often not affected, and caterpillars remain alive and can eventually damage an equivalent number of kernels. In addition, "railroading" (where small larvae slightly damage many kernels along a silk channel without consuming them) occurs at varying rates on milk stage kernels in the Bt hybrids expressing high levels of the protein in kernels (Dowd 2000, current study) compared with almost no occurrence in no/low kernel expression Bt hybrids. This feeding pattern usually does not occur until kernels become relatively dry (Dowd, unpublished data). It is possible that the antifeedant effects of the Bt protein (Rajedran et al. 1994, Berdegue et al. 1996) are responsible for this behavior, although poor nutrient absorption that mimics starvation may also contribute.

The hybrids that express the gene at low levels in the ear and silk are more variable in reducing caterpillar damage. This appears to be related to the timing of invasion of the ears. Past reports have indicated when *O. nubilalis* invasion occurs before kernel fill, some significant control will occur for NKMax 454 versus NK4494 (Dowd 2000). However, in most cases, when invasion occurs after some kernel fill has occurred, there is no significant difference for incidence–numbers of kernels damaged per ear (Dowd 2000, current study). This phenomenon appears to be the result of greater toxicity of the unfilled kernels than the filled kernels, because of a differential expression pattern of the gene construct for event 176 hybrids (Dowd 2000).

In the current study, there was only one case (Manito 1998) where caterpillar control in general was much better ultimately for a low kernel expression Bt versus non Bt hybrid. This appears to be caused by a large but late invasion of caterpillars. Invasion was late enough so that feeding at the ear tip was undesirable because of wilted or dry silks, and instead, insects fed on the husk before entering the ear. Incidence and feeding were reduced on the NKMax454 ears at milk stage relative to the non-Bt hybrid at Manito similar to that typically noted for high expressing Bt hybrids. In some cases, husk feeding on the NKMax454 ears at Manito was also noted, but damage did not penetrate to kernels.

Indirect Reduction of Mycotoxins. Past studies have indicated that reducing insect damage in Bt hybrids can significantly reduce fumonisin levels compared with corresponding non-Bt hybrids in small plot (Munkvold et al. 1999, Dowd 2000), and larger fields under natural conditions (Dowd 2000). Greater differences between Bt and non-Bt hybrids have been noted in small-plot studies when ears are inoculated (Munkvold et al. 1999, Dowd 2000). High levels of reductions can occur in the high Bt hybrids when only *O. nubilalis* is present (Munkvold et al. 1999, Dowd 2000). However, when *H. zea* is more common, reductions in fumonisins are more limited in Bt versus non-Bt hybrids (Dowd 2000, current study). This reduction in efficacy would be expected because an association between *H. zea* damage and *Fusarium* ear molds in corn has been known for some time (e.g., Smeltzer 1959). Those hybrids that express only low levels of Bt in kernels and silks are generally less effective in indirectly reducing fumonisin levels relative to hybrids that express high levels in kernels (Munkvold et al. 1999, Dowd 2000).

Levels of fumonisins in NKMax454 were higher than for NK6800Bt and P33V08 in previous studies in 1997 and 1998 as well (Dowd 2000). Interestingly, in several cases in the current study, there were higher correlations between insect damage and fumonisin levels than between symptomatic mold infected kernels and fumonisin levels. This information suggests that the mold is still present asymptomatically in the insect-damaged kernels or has spread from insect damaged kernels asymptomatically into other kernels. The Bt inbreds (Dowd 2000) and hybrids (Williams et al. 1999, Wilson et al. 1999, Windham et al. 1999, Dowd 2000) are generally much less effective in reducing *A. flavus* or aflatoxins. Environment– hybrid interactions appear to be influencing efficacy of indirect reductions of mycotoxins in the case of both fumonisins and aflatoxins.

Hybrid–Environment Influences. Past studies involving chemical insect control as a means for indirectly reducing mycotoxins such as aflatoxin have shown variation in efficacy for both different hybrids in the same year, and the same hybrids at different years or at different sites (review Dowd 1998). These types of situations have been reported for corn and aflatoxins (e.g., Anderson et al. 1975, Lillehoj et al. 1976). A similar situation appears to occur for fumonisins as well. Studies using insecticide treatments for indirect reduction of fumonisins have also noted differences in efficacy between hybrids in the same year, and between the same hybrid in different years (Dowd et al. 1999b). The degree of correlation between insect damage and fumonisin levels varied in the same manner (Dowd et al. 1999b).

Significant correlations between insect damage and fumonisin levels have been noted in the past for Bt and non-Bt hybrids (Munkvold et al. 1999, Dowd 2000). These correlations also indicate Bt hybrids, which reduce insect damage, also can produce corresponding reductions in fumonisins (Munkvold et al. 1999, Dowd 2000). Significant correlations between insect-damaged kernels and fumonisins have been noted in some years but not others for NK4494/NKMax454 in 0.4-ha fields (Dowd 2000) and in larger areas (current study). Similarly, significant correlations between insect damage and fumonisin levels were noted between P3394/33VO8 pair in 1997 and 1998 (Dowd 2000), but not 1999 (current study). The NK6800/6800Bt hybrid pair has shown significant associations between insect damage and fumonisins in 0.4-ha fields in 1997 and 1998 (Dowd 2000). In the current study, the NK6800Bt (only one of the pair available) also showed significant correlations between insect damage and fumonisin levels in larger commercial fields in 1998 and 1999 at different sites. Thus, it is possible that some hybrids will be more consistently useful in indirectly reducing ear mold levels than others. However, as indicated by the current study, even hybrids from the same company will vary in the degree of correlation between insect damaged kernels and mycotoxin levels. Although NK6800Bt proved very consistent, at the same site in 1998, NK7639Bt (another Bt11 event hybrid) showed significantly higher incidence of ears with symptomatically *Fusarium*-infected kernels, and significantly more infected kernels per ear, in spite of no significant differences in incidence of insect damage or numbers of insect damaged kernels per ear.

This varying relationship can be understood better if one considers the interaction of environment–insects– ear molds (see discussion in Dowd 1998). A favorable environment for the mold will increase the amount of inoculum and duration of inoculum viability. An unfavorable environment for the plant will decrease the defensive response against the mold. Through vectoring and damage, the insect can overcome plant barriers to the mold that otherwise may be difficult for the mold to overcome. Once established on an insect-damaged kernel, the mold may or may not be able to readily spread to adjacent, undamaged kernels, again as influenced by the natural defenses of the plant and how the abiotic environment affects them.

In conclusion, it is difficult and often impractical to obtain significant reductions in mycotoxins by controlling insects with insecticides (review Dowd 1998, 1999b). However, Bt hybrids can virtually eliminate *O. nubilalis* damage (Munkvold et al. 1997, Pilcher et al. 1997b, Dowd 2000), and great reductions in fumonisins have been seen (Munkvold et al. 1999, Dowd 2000, current study). In contrast to conventional formulations of insecticides, beneficial insect predators are generally not affected by Bt corn in the field (e.g., Pilcher et al. 1997a, Dowd 2000, current study). When *O. nubilalis* is the predominant pest, the potential exists to greatly reduce fumonisin levels in commercial fields, provided the fungus does not invade readily on its own. Again, successful invasion of the fungus may be further dependent on environmental conditions that favor the fungus and/or interfere with plant defensive capabilities. In addition, when other insects such as *H. zea* or sap beetles are present, the number of damaged kernels present may still result in some fumonisin contamination. However, Bt corn, especially those hybrids that express the protein throughout the plant (kernels and silk), is likely to help prevent undesirable levels of fumonisin. Considering that *H. zea* are usually uncommon in the midwestern corn belt, mycotoxin reductions are likely to be more significant in the Midwest, where *O. nubilalis* causes a higher proportion of ear damage but can be effectively controlled by Bt corn that expresses high levels of the protein in the kernels and silks, than in other parts of the United States where *H. zea* (which is less effectively controlled by Bt corn) is more common. Newer hybrids with high resistance to *H. zea* and sap beetles should help eliminate these sources of mycotoxins as well.

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